

APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

TITLE: HS-40 ENHANCER-CONTAINING VECTOR

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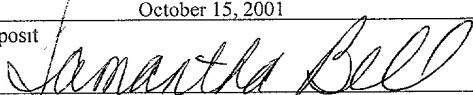
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HS-40 ENHANCER-CONTAINING VECTORBackground of the Invention

5 HS-40 is a 350-400 bp enhancer element located about  
40 kb upstream of  $\zeta$ -globin gene, which is expressed in the  
human embryonic erythroblasts but not in the human adult  
erythroblasts. Specific elements within the HS-40 enhancer  
have been identified, including GATA-1 motifs, NF-E2/AP1  
10 motifs (a 3' and a 5' motif), and a Sp1 binding site.

Summary of the Invention

The invention is based on the discovery that a  
single nucleotide change in the 3'NF-E2/AP1 element of the  
human HS-40 enhancer, unlike the wild type HS-40 enhancer,  
15 confers position-independent and copy number-dependent  
expression on a transgene. In addition, the single  
nucleotide change allows expression of the gene in the cells  
of an adult mouse, an effect not seen for the wild type  
HS-40 enhancer.

Accordingly, the invention features a viral  
expression vector (e.g., a retrovirus) having a nucleic acid  
including (1) a transcriptional start site; (2) a promoter  
(e.g., a tissue-specific promoter such as a  $\zeta$ -globin  
promoter) operably linked to the transcriptional start site;  
20 and (3) an enhancer operably linked to the promoter, the  
enhancer including the mutated NF-E2/AP1 (mtNF-E2/AP1) DNA  
sequence TCTGAGTCA (SEQ ID NO:1) or the RNA equivalent  
thereof. The underlined "T" represents a mutation of the  
wild type "G" in the wild type NF-E2/AP1 (wtNF-E2/AP1)  
25 sequence. In a specific embodiment, the enhancer includes  
the minimal mutated HS-40 DNA sequence

AGATAACTGGGCCAACCATGACTCAGTGCTCTGGAGGCCAACAGGACTTCTGAGTCATC  
CTGTGGGGTGGAGGTGGACAAGGGAAAGGGTGAATGGTACTGCTGATTACAACCTCT  
GGTGCTGCCTCCCCCTCCTGTTATCT (SEQ ID NO:2)

or an RNA equivalent thereof. The bold sequence represents  
5 the mtNF-E2/AP1 site with the G to T mutation underlined.  
The minimal HS-40 enhancer sequence excludes a 5' GATA-1(b)  
site because it has been shown that this site is not  
necessary for HS-40 enhancer activity (Zhang et al., J Biol  
Chem 270:8501-8505, 1995).

10 The enhancer can also include the full mutated HS-40  
enhancer sequence:

TCGACCCTCTGGAACCTATCAGGGACCACAGTCAGCCAGGCAAGCACATCTGCCAAGCC  
AAGGGTGGAGGCATGCAGCTGTGGGGTCTGTGAAAACACTGAGGGAGCAGATAACTGG  
GCCAACCATGACTCAGTGCTCTGGAGGCCAACAGGACTTCTGAGTCATCCTGTGGGGT  
GGAGGTGGACAAGGGAAAGGGTGAATGGTACTGCTGATTACAACCTCTGGTGCCTGCCT  
CCCCCTCCTGTTATCTGAGAGGGAAGGCCATGCCAAAGTGTTCACAGCCAGGCTTCAG  
GGGCAAAGCCTGACCCAGACAGTAAATACGTTCTTCATCTGGAGCTGAAGAAATT  
(SEQ ID NO:3)

or an RNA equivalent thereof. The bold sequence represents  
20 the mtnf-E2/AP1 site with the G to T mutation underlined.  
This sequence is referred to herein as the mHS-40 sequence,  
which differs from the wild type HS-40 (wHS-40) sequence by  
the G/T mutation indicated above. Again, the single  
mutation is underlined. The vector can also contain a  
25 transcriptional termination signal (e.g., a polyadenylation  
signal). In other embodiments, the promoter drives  
transcription of a mRNA encoding a polypeptide (e.g., a  
growth hormone), the transcription beginning from the  
transcriptional start site.